March 12, 2001

MEMORANDUM

SUBJECT: **LACTOFEN**: Report of the Mechanism of Toxicity Assessment Review Committee

FROM: Robert F. Fricke
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THROUGH: Karl Baetcke
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and

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EPA Identification Nos: PC Code: 128888
DP Barcode: D267472
Submission: S582396
Case: 819544

Action Requested: The Registrant (Valent U.S.A) has submitted a petition (MRID No. 45160301) requesting that risk assessment for lactofen be based on the MOE approach rather than using a Q_1* of 0.119 $(mg/kg/day)^{-1}$ in human equivalents (HED Doc No.: 014237, July 12, 2000). The petition reviewed and summarized earlier data submissions, which supported peroxisome proliferation as the mechanism of action of lactofen.

Conclusions: On January 17, 2001, the Mechanism of Toxicity Assessment Review Committee (MTARC) reviewed the merits of the toxicological data supporting peroxisome proliferation as the proposed mode of action for lactofen. Based on the weight-of-evidence from guideline, as well as mechanistic studies ,the MTARC concluded that the evidence presented on peroxisome proliferation indicates that lactofen operates via a mode of action involving PPAR activation.

Evaluation Criteria: Based on the International Life Sciences Institute (ILSI) recommendations, the following criteria were used to assess the mode of action of lactofen:

1.) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis.

2.) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

3.) Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities.

Weakness of Submitted Data Supporting Mode of Action on Peroxisome Proliferation:

The only weakness in the submitted data was that BrdU labeling was not performed to evaluate replicative DNA synthesis. The MTARC concluded that this shortcoming did not impact on their conclusions; ample evidence was submitted which demonstrated hepatic hyperplasia after treatment with lactofen.

Strengths of the Submitted Data in Supporting Mode of Action on Peroxisome Proliferation:

1.) The results guideline studies showed that lactofen is neither mutagenic nor genotoxic. A non-guideline study showed equivocal (probably negative) binding of lactofen to DNA.

2.) Changes in liver morphology were observed in both rats and mice treated with lactofen. These effects include: dose-dependent increase in relative liver weights and increased number of peroxisomes as measured by electron microscopic analysis. Further, in mice there was a dose-dependent increase in nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining.

3.) There was evidence of cell proliferation as measured by increased relative liver weights and histological evidence indicating hepatic hyperplasia. These effects were dose-related with a clearly defined threshold.

4.) Dose-dependent increases (particularly in female mice) in the activities of hepatic CN-insensitive palmitoyl CoA oxidase and carnitine acetyl transferase. Dose-dependent increase in

CN-insensitive palmitoyl CoA oxidase was also observed in primary rat hepatocytes treated with lactofen.

5.) The doses at which carcinogenicity was observed (mouse, LOAEL = 50 ppm; rat, LOAEL = 2000 ppm) were consistent or higher than the doses which caused peroxisome proliferation.

Other Issues Discussed:

The MTARC recommended that the cancer classification be reviewed by the Cancer Assessment Review Committee. Since the toxicological database for lactofen is relatively extensive, the MTARC further recommended that the Registrant submit the lactofen studies to ILSI as a case study.

CC: Elizabeth Mendez (HED, 7509C)
Christina Scheltema (SRRD, 7508C)

Assessment of the Mode of Action for Lactofen

I. Background

A joint meeting (March 29, 2000) of the Mechanism of Toxicity Assessment Review Committee (MTARC) and Cancer Assessment Review Committee (CARC) evaluated the adequacy of the toxicological database for diclofop-methyl in support of peroxisome proliferation as the mechanism of action for liver carcinogenicity. The basis for the evaluation was two detailed literature reviews on the role of peroxisome proliferation in hepatocarcinogenesis. One review [1] by the International Life Sciences Institute (ILSI) evaluated the human cancer risk of peroxisome proliferation, while the other [2] evaluated the human cancer risk of the plasticiser di(2-ethylhexyl)phthalate (DEHP).

As a result of this joint meeting of the MTARC and CARC, criteria (based on the ILSI recommendations) were established which should be met before a non-genotoxic hepatocarcinogenic substance can be classified as a peroxisome proliferator (PP). These criteria are:

1.) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis.

2.) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

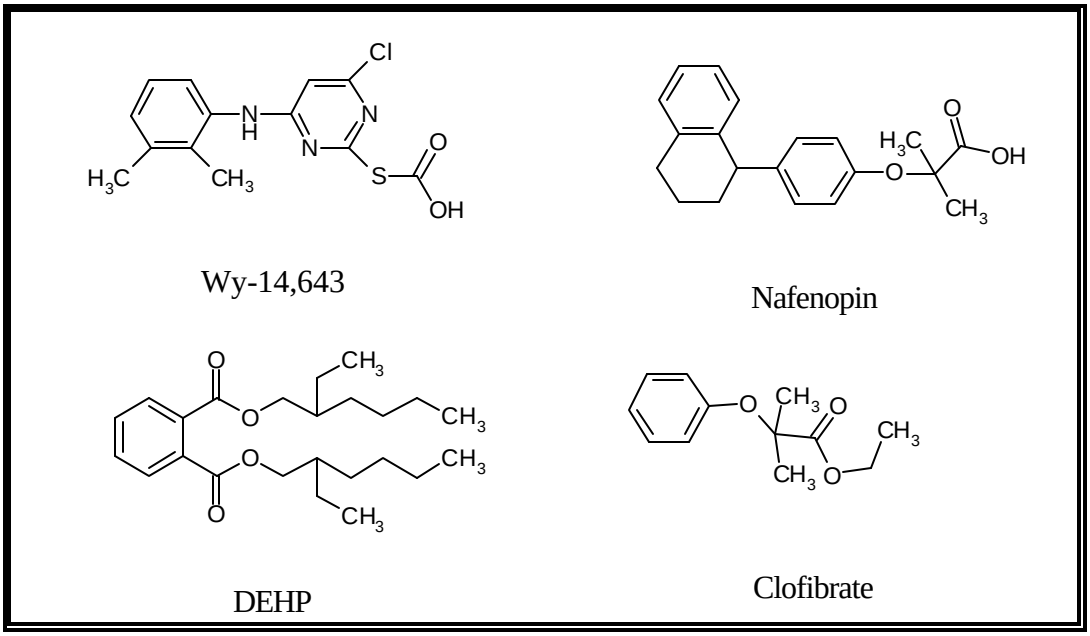
3.) Increased activity of enzymes involved in peroxisomal fatty acid metabolism, especially acyl (palmitoyl) CoA oxidase activities.

II. Mechanism of Action of Peroxisome Proliferators

PPs are a diverse group of chemicals and include synthetic (industrial plasticisers and solvents and hypolipidemic drugs), as well as the naturally occurring compounds (certain fatty acids, prostaglandins, and steroids). Although the synthetic PPs are structurally dissimilar, they share a similarity with fatty acids - a large hydrophobic region and an aliphatic chain with a terminal acidic group, usually carboxylic acid (Figure 1).

Research over the past decade showed that the rodent is highly responsive to PPs and that the liver is the primary target organ. The action of PPs on rodent liver leads to specific and well characterized toxicological events. Short-term effects (as early as one week) on the liver include hepatomegaly due to hypertrophy and hyperplasia, increase in the number and size of peroxisomes, and the transcriptional induction of peroxisomal enzymes (acyl CoA oxidase), endoplasmic reticulum (cytochrome P450) and cytosol (fatty acid binding protein). While these short-term effects on the liver are reversible, long-term exposure leads to the development of hepatic cancer.

Figure 1: Chemical Structures of Some Peroxisome Proliferators



A receptor-based mechanism for the proliferation of peroxisomes was established with the identification of peroxisome proliferator-activated receptor á (PPARá) [3], which is a member of the nuclear receptor superfamily. The binding of PPARá to the retinol X receptor (RXR) produces a heterodimer which becomes fully active with the binding of a PP to the active site. The active PPARá-RXR-PP complex is capable of regulating gene expression through interaction with the peroxisome proliferator responsive elements (PPRE) of target genes.

In a recent review[4], the large number of PPs preferentially activate PPARá; several hypolilidemic drugs (clofibrate) and Wy-14,643 have been shown to directly bind to PPARá. They further point out that the ability of a PP to bind to and/or activate PPARá correlated well with the hepatocarcinogenic potency of the PP. Further definitive evidence for the involvement of PPARá in the development of hepatocellular tumors was demonstrated using transgenic mice which lacked expression of PPARá mRNA.

The introduction of PPARá null mice(-/-) by Lee et al.[5], the obligatory role of PPARá in mediating the cellular effects of PP was established. Short-term exposures to Wy-14,643 or clofibrate showed that while wild-type (+/+) mice developed the classic hepatic effects, the effects in treated null (-/-) mice were comparable to the (-/-) controls. In a chronic toxicity study [6] (+/+) and (-/-) mice were fed either basal diet or diet containing 0.1% Wy-14,643. After one week of treatment, the (+/+) mice had a significant increase (93%) in relative liver weight; further increases in relative liver weights were observed at 5 weeks (245%) and 11 months (355%) of treatment. By contrast, the relative liver weights of treated (-/-) mice were comparable to control (-/-) mice at all timepoints. To evaluate replicative DNA synthesis, the BrdU labeling index was measured in both (+/+) and (-/-) mice. While the BrdU labeling index was significantly increased in (+/+) mice after 1 and 5 weeks of treatment compared to (+/+) controls, the BrdU labeling indexes of treated (-/-) mice were comparable to (-/-) controls at these same timepoints. Gross examination revealed multiple visible nodules in the livers of

treated (+/+) mice; microscopic examination showed a 100% incidence of hepatocellular neoplasms. The livers of the treated (-/-) mice were grossly and microscopically comparable to (-/-) control mice.

These data firmly established the central, and necessary, role of PPARá in nongenotoxic hepatocarcinogenesis.

III. Evaluation of Toxicity Database for Lactofen

Both guideline and non-guideline (mechanistic) studies have been reviewed. Pertinent results of guideline studies relevant to the proposed mechanism of action of lactofen are summarized in Table 1.

A. Subchronic Toxicity Studies with Lactofen

1) 4-Week Feeding Study in the Rat (MRID No: 00117563): In a 4-week range-finding study, rats were fed diets at 0, 200,100, 5000 or 10000 ppm lactofen (76% a.i.). Animals in the high-dose group had 100% mortality by day 7. Increased liver and kidney weights were observed at doses of 1000 ppm and higher.

2) Subchronic Feeding Study in the Rat (MRID No: 00117564): In a 90-day subchronic toxicity study, rats were fed diets containing 0, 40, 200 or 1000 ppm lactofen (males: 0, 2.9, 14, 74 mg/kg/day; females: 0, 3.5, 17, or 85 mg/kg/day). At the high-dose level, increased relative (to body weight) liver weights in males (3.4%, control 2.8%) and females (3.1%, control 2.7%) and absolute liver weights in males (18.4 g, control 15.0 g) were observed. Gross findings were observed only in high-dose animals and included dark livers in high-dose males (15/19) and females 4/21); darkened renal cortex was also observed in high-dose males (15/19) and females (3/21). Histopathological evaluation of high-dose animals revealed brown pigmentation in hepatocytes and/or Kupffer cells (males, 17/19; females, 7/21; none in controls), and acidophilic hepatocellular degeneration in males (10/19, none in control) and females (1/21, none in controls) and hyperplasia of bile ducts in males (6/19; controls, 1/20). Other microscopic lesions included brown pigment in tubular epithelium in the kidneys of high-dose animals (males, 12/19; females, 3/21; none in controls).

3) Subchronic Feeding Study in the Mouse (MRID No: 00132882): In a 90-day subchronic toxicity study, mice were fed diets containing 0, 40/2000, 200, 1000, 5000, or 10000 ppm lactofen (calculated doses: 0, 5.7, 29/286, 143, 714 or 1429 mg/kg/day). Further as a result of 100% mortality in 5000 and 10000 ppm males and females, the 40 ppm dose was increased to 2000 ppm at week 5. With the exception of one female, all of the 2000 ppm animals died between 5 and 10 weeks after the increase in the dose. Dose-related increases in serum cholesterol and total protein levels, as well as liver enzymes (ALP, ALT and AST) were observed in 200 and 1000 ppm males and females. Comparable increases in absolute and relative liver weights were seen at 200 ppm (males, 43 - 48%; females, 45 - 53%) and 1000 ppm (males, 198 - 221%; females, 189 - 190%). Gross examination revealed enlarged livers in 200, 1000 and 2000 ppm mice and enlarged spleens in 1000 and

2000 ppm mice. At 2000 and/or 1000 ppm, microscopic examination of the liver showed hepatocytic vacuolization, necrosis of individual hepatocytes, bile retention, coagulative necrosis, hyperplasia of biliary epithelium and increased extramedullary hematopoiesis; the kidney showed nephrosis and cortical fibrosis/scarring.

B. Chronic Toxicity, Carcinogenicity and Reproductive Toxicity Studies with Lactofen

1) Combined 2-Year Chronic Feeding/Carcinogenicity Study in the Rat (MRID No.: 00150329): In this study, rats were fed diets containing 0, 50, 500, 1000, or 2000 ppm (0, 2, 19, 38, and 76 mg/kg/day, based on 20 ppm = 1 mg/kg) lactofen for 104 weeks. Effects seen at 1000 ppm included increased incidence of mottled diffusely dark livers and kidneys, increased aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities, decreased cholesterol, blood urea nitrogen, and total protein globulin levels, and increased incidence in the pigmentation of hepatocytes, Kupffer cells and renal cortical tubule cells. Effects seen at the 2000 ppm were similar to those seen at 1000 ppm, but more severe. Other effects at 2000 ppm included increased incidence in basophilic or eosinophilic foci of cellular alteration and increased incidence of neoplastic liver nodules.

2) 18-Month Carcinogenicity Study in the Mouse (MRID No. 00150343): In this study, mice were fed diets containing lactofen at 0, 10, 50, or 250 ppm (0, 1.4, 7.1, or 36 mg/kg/day, based on 1 ppm = 0.143 mg/kg) for 78 weeks. Effects seen at 50 ppm included increased liver weight, increased incidence of dark colored and/or enlarged livers, hepatocytomegaly (also observed in males at 10 ppm); increased incidences of focal cell alteration (females only), and hepatocellular adenomas ; sinusoidal cell pigmentation in the liver was observed in all dose groups. At the highest-dose tested (250 ppm), the severity of these signs of toxicity was increased. Other effects noted at 250 ppm included increased incidence of non-neoplastic and neoplastic liver masses and increased kidney pigmentation.

3) Chronic Feeding Study in the Dog (MRID No: 41967901): In a one-year toxicity study, dogs were fed diets containing 0, 40, 200, or 1000/3000 (0, 0.79, 4.0, 20/59 mg/kg/day based on 1 mg/kg = 40 ppm) lactofen; because of lack of significant toxicity at 1000 ppm, the dose was increased to 3000 ppm after 4-months of treatment. Dogs fed the 1000/3000 ppm diet showed a slight increase in peroxisomal staining (based on the intensity of brown stippling of D.A.B. stained slides) in the livers. Relative liver weight was increased in high-dose females. There was no evidence of nuclear enlargement, increased mitotic activity, inflammation, or focal necrosis.

Table 1: Summary of Guideline Toxicity Studies with Lactofen

Study (MRID No.)	Liver Weight	Liver Enzymes	Liver Histopathology
4-Week Feeding - Rat (00117563) 0, 200, 1000, 5000, 10000 ppm 100% lethality at 10000 ppm	5000 ppm **%%** and **&&**: Increased relative wt	Not measured	Not measured
13-Week Feeding - Rat (00117564) 0, 40, 200, 1000 ppm **%%**: 0, 2.9, 14, 74 mg/kg/day **&&**: 0, 3.5, 17, 85 mg/kg/day	1000 ppm: Increased abs and rel in **%%** and rel in **&&**	1000 ppm **%%**: increased ALT, AST and ALK	1000 ppm, **%%** and **&&**: Brown pigmentation in hepatocytes and/or Kupfer cells and acidophilic hepatocellular degeneration.
13-Week Feeding - Mice (00132882) 0, 40/2000, 200, 1000, 5000, 10000 ppm 0, 5.7/286, 29, 143, 714 mg/kg/day 40 ppm increased to 2000 ppm at wk 7 100% lethality at HDT	$200 ppm **%%** and **&&**: Increased relative and absolute wt	$200 ppm: Increased ALK in **%%** and **&&**, increased ALT and AST in **%%** $1000 ppm: Increased ALT and AST in **&&**, increased total cholesterol in **%%** and **&&**	$200 ppm: Enlarged liver in **%%** and **&&**, extramedullary hematopoiesis in **&&**, $1000 ppm **%%** and **&&**: Hepatocellular vacuolation, bile retention, hepatocellular swelling, and extramedullary hematopoiesis $1000 ppm **%%**: Necrosis of individual hepatocytes,
Carcinogenicity - Mouse (00150343) 0, 10, 50 , 250 ppm 0, 1.4, 7.1, 36 mg/kg/day	$ 10 ppm **%%**, $50 ppm **&&**: Increased abs and rel wts	Not measured	Hepatomegaly: $10 ppm **%%** and $50 ppm **&&** Focus/Area of Cell Alteration: 50 ppm **%%** and 250 ppm **&&** Sinusoidal cell pigmentation.: $10 ppm in **%%** and **&&** Necrosis of individual hepatocytes: 250 ppm **%%** Hepatocellular Adenoma: $50 ppm **%%** and **&&** Carcinoma: 250 ppm **%%** and **&&** Combined: 250 ppm **%%** and **&&**

Study (MRID No.)	Liver Weight	Liver Enzymes	Liver Histopathology
2-year Combined Chronic/Onco - Rat (00150329) 0, 50, 500, 1000, 2000 ppm 0, 2, 19, 38, 76 mg/kg/day	$1000 ppm, **%%**, $500 ppm **&&**: Increased relative wt	$500 ppm, **%%** and **&&**: Increased AST, ALT and ALK at 18 mo.	$1000 ppm,: Pigmentation in hepatocytes(**&&** only) and Kupfer cells (**%%** and **&&**) 2000 ppm: Increased basophilic and eosinophylic (**%%** only) foci of cellular alteration and proliferative nodules
1-Year Chronic Feeding Study - Dog (41967901) 0, 40, 200, and 1000/3000 ppm 0, 0.8, 4, 20/59 mg/kg/day	1000/3000 ppm **&&**: Statistically significant increases in the relative liver weight; absolute liver weight not affected.	Not measured	No histopathological findings observed in the liver
2-Generation Reproduction - Rat (00132885) 0, 50 500, 2000 ppm	<u>F1 Adults</u> $500 ppm **&&** and 2000 ppm **%%**: Increased relative wt 2000 ppm **&&**: Increased absolute wt. <u>F1 Pups</u> $500 ppm **%%**: Increased absolute wt 2000 ppm **&&**: Increased relative wt.	Not measured	<u>F1 Adults</u> 2000 ppm **%%** and **&&**: Centrilobular necrosis/degeneration, brown pigmentation [hemosiderin] in hepatocytes and reticulo-endothelial cells

4) Reproductive Toxicity Study in the Rat (MRID No: 00132885): In this study, rats were fed diets containing lactofen at 0, 50, 500, or 2000 ppm (F0 males/females: 0, 2.6/3.1, 26.2/31.8, 103.5/121.3 mg/kg/day; F1 males/females: 0, 2.7/3.3, 26.732.9, or 115.4/138.9 mg/kg/day). For parental groups at the high-dose level, there was increases in spleen and liver weights; increased incidence of liver [hepatocytic centrilobular degeneration and necrosis] and spleen [extramedullary hematopoiesis] microscopic lesions. For offspring groups no liver toxicity was noted

C. Mutagenicity and Genotoxicity Studies with Lactofen

The guideline and non-guideline studies indicate that lactofen is neither genotoxic nor mutagenic. Equivocal (probably negative) results were observed in an *in vivo* DNA binding assay with radiolabeled lactofen. Results are summarized in Table 2.

Table 2: Summary of Mutagenic and Genotoxic Effects of Lactofen

Assay	Results
Salmonella typhimurium/mammalian microsome mutagenicity assay (MRID No.00150346, 00150346)	Negative: ± S9 including
Salmonella typhimurium/mammalian microsome mutagenicity assay (MRID No. 00150347)	Negative ± S9
In vitro cytogenetic assay with Chinese hamster ovary (CHO) cells (MRID No. 00150348)	Negative for clastogenic effects ± S9
In vitro cytogenetic assay with Chinese hamster ovary (CHO) cells (MRID No. 00150626)	Negative for clastogenic effects ± S9
In vitro unscheduled DNA synthesis in primary mouse hepatocytes (MRID No. 00150349 and 00162141)	Negative
In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID No. 00150627)	Negative
In vivo DNA covalent binding in mouse liver (MRID No. 00150350)	Equivocal (probably negative) Low level of binding

D. Mechanistic Studies with Lactofen

Several non-guideline *in vitro* and *in vivo* mechanism studies have been submitted which further characterized lactofen-induced peroxisome proliferation. These studies include the measurement of biochemical markers for peroxisome proliferation in the mouse and rat, as well as in primary rat hepatocytes, and a DNA binding study in the mouse. A summary of the results of these mechanistic studies is summarized in the Table 3.

Table 3: Summary of Results for Mechanistic Studies with Lactofen

Study (MRID No)	**Liver Weights**	**Enzyme**	**Pathology/histopathology** **Electron Microscopy**
Analysis of biochemical and microscopic parameters in Chimpanzee liver (45283901, 45283905) 5 and 75 mg/kg/day	Not measured	Aryl CoA oxidase, catalase and carnitine acetylytransferase activities not affected by treatment	No nuclear enlargement, cytoplasmic eosinophilia or hepertrophy observed in liver biopsies after 0, 1, and 3 months of treatment Slight + response for peroxisomal staining (brown stippling)
Results of the Analysis of Biochemical Parameters in Mouse and Rat Liver Following Exposure to PPG-844 (45283904) Mouse: 0, 2, 10, 50, 250 technical 250 ppm pure Rat: 0 and 2000 ppm technical	Mice at 7-Weeks: $10 ppm%% and $50 ppm&& Increased rel wt	Catalase and CN-insensitive palmiloyl CoA oxidase increased in%% at 250 ppm and&& at $50 ppm.	Pathology: Rats (2000 ppm) and mice ($50 ppm) showed increased nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomes in number of peroxisomes. EM: Rats (2000 ppm) and mice (250 ppm) showed quantitative increase in the number of peroxisomes
Measurement of Peroxisome Proliferation in Primary Rat Hepatocytes Induced by PPG-844 and Five of its Metabolites (45283902)	Not applicable	Concentration-dependent increase in CN-insensitive palmitoyl CoA oxidase activities with each of the metabolites.	EM: Lactofen (0.01 mM) increased number of peroxisomes and glycogen aggregates. Other metabolites showed occasional peroxisomes

1) Subchronic Toxicity Study in Male Chimpanzees (MRID No.: 45283901, 45283905): In this study, three male chimpanzees per dose group were orally dosed with lactofen at 5 and 75 mg/kg/day for three months, followed by a two-month recovery period. Whole blood was collected before and during treatment for clinical pathological evaluations; liver biopsies were obtained before and after 30 and 90 days of treatment. Compared to pre-treatment values, the activities of acetyl CoA oxidase, catalase and carnitine acetyl transferase in the liver were not affected by treatment. Histopathological evaluation of the liver biopsies did not show any evidence of nuclear enlargement, cytoplasmic eosinophilia, or hypertrophy; peroxisome content in the liver biopsies did not show any change in the pre- and post-treatment evaluations. Further, electron microscopic evaluation of liver did not reveal any evidence of peroxisome proliferation. Based on the results of this study lactofen was not a peroxisome proliferator in chimpanzees.

2) Measurement of Biochemical and Histopathological Markers for Peroxisome Proliferation in Rat and Mouse Livers (MRID No.: 45283904): In a dietary study male and female Crl:CD(S)Br rats and Crl:CD1 mice were fed diets containing lactofen or nafenopin, a positive control for peroxisome proliferation. Biochemical markers of peroxisomal proliferation included measurement of hepatic acyl CoA oxidase, catalase and carnitine acetyl transferase activities. Light and electron microscopic were used to evaluate livers for evidence of peroxisome proliferation

Male and female mice were exposed to technical lactofen at 0, 2, 10, 50, or 250 ppm, pure lactofen at 250 ppm, or nafenopin, at 500 ppm. After 7 weeks of treatment, male and female mice showed significant biochemical and pathological effects on the liver. Dose-dependent increases in relative liver weights, catalase and acyl CoA oxidase were observed in males and females; females also showed a significant increase in carnitine acetyl transferase (Table 4). Liver histology (Table 5) revealed significant, dose-dependent increases in nuclear enlargement, cytoplasmic eosinphilia, hypertrophy and peroxisomal staining. Nafenopin-treated mice showed significant increases in all of the parameters measured.

Similar findings were also observed in an 8-week study, in which rats were fed diets containing lactofen at 0 or 2000 ppm or nafenopin at 500 ppm. Lactofen-treated rats had significantly increased relative liver weights, carnitine acetyl transferase and acyl CoA oxidase (Table 6). Histological examination revealed increased incidence of nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining (Table 7); catalase activity was not affected by treatment. Nafenopin-treated rats showed significant increases in all of the parameters indicative of peroxisome proliferation

Electron microscopic analysis was performed on the livers of male rats and mice. As a quantitative measurement of peroxisome proliferation, the ratio of peroxisomes to mitochondria were determined. The ratios were 1/4.7 (21%) and 1/3.0 (33%) for control and treated (2000 ppm, 8 weeks) male rats, respectively, and 1/4.8 (21%) and 1/1.5 (66%) for control and treated (250 ppm, 7 weeks) mice, respectively.

Table 4: Evaluation of Liver Parameters in Male and Female Mice After 7 Weeks of Treatment with Lactofen (Technical, 78.2%; pure, 99.8%) or Nafenopin (NAF)

Sex	Dose (ppm)	Relative Liver Wt (g/100 g)	Catalase	Carnitine Acetyl Transferase	Palmitoyl CoA Oxidase
Male	0	3.9	0.34	2.92	3.1
	2 (Tech)	4.2	0.49	2.96	5.2
	10 (Tech)	5.0***	0.41	3.87***	6.1
	50 (Tech)	4.5**	0.49	3.27	7.2
	250 (Tech)	7.7***	0.94***	3.1	40***
	250 (pure)	6.2***	0.93***	6.77***	29***
Female	0	4.0	0.22	3.21	2.1
	2 (Tech)	4.1	0.26	3.71	4.3
	10 (Tech)	4.4	0.21	4.27**	11***
	50 (Tech)	4.7**	0.30*	4.68***	13***
	250 (Tech)	6.7***	0.69***	4.53**	35***
	250 (pure)	7.3***	00.44***	7.77***	27***
Male	500 NAF	12.8***	0.70***	8.31***	49***
Female	500 NAF	11.9***	0.76***	9.94***	38***

** p # 0.01; *** p # 0.001

[1] Data summarized from Tables 5 and 6 of MRID No. 452383904.

Table 5: Evaluation of Histopathological Parameters in Male and Female Mice After 7 Weeks Treatment with Lactofen (Technical, 78.2%; Pure, 99.8%) or Nafenopin (NAF) [1]

Sex	Dose (ppm)	Nuclear Enlargement	Cytoplasmic Eosinphilia	Hypertrophy	Peroxisomal Staining
Male	0	!	!	!	+
	2 (Tech)	!	!	!	+
	10 (Tech)	±	±	±	+
	50 (Tech)	+	+	+	++
	250 (Tech)	+	++	++	++
	250 (Pure)	++	+++	++	++
Female	0	!	!	!	+
	2 (Tech)	!	!	!	+
	10 (Tech)	±	!	!	+
	50 (Tech)	+	+	+	++
	250 (Tech)	++	++	++	+++
	250 (Pure)	++	+++	+++	++
Male	500 NAF	++	+++	+++	+++
Female	500 NAF	+++	+++	+++	+++

[1] Data summarized from Table 7 and 8 of MRID No. 452383904.

Table 6: Evaluation of Liver Parameters in Male and Female Rats After 8 Weeks of Dietary Treatment with Lactofen (Technical, 78.2%) or Nafenopin (NAF)

Sex	Dose (ppm)	Relative Liver Wt (g/100 g)	Catalase	Carnitine Acetyl Transferase	Palmitoyl CoA Oxidase
Male	0	3.0	0.37	3.5	3.4
	2000	4.3***	0.36	6.4***	8.6**
Female	0	2.8	0.20	7.3	5.3
	2000	3.8***	0.19	2.4***	18.3***
Male	500 NAF	5.4***	0.60***	2.9	13.3***
Female	500 NAF	3.7***	0.30***	10.2***	12.5**

** p # 0.01; *** p # 0.001

[1] Data summarized from Table 11 of MRID No. 452383904.

Table7: Evaluation of Histopathological Parameters in Male and Female Rats After 8 Weeks Treatment with Lactofen (Technical, 78.2%) or Nafenopin (NAF) [1]

Sex	Dose (ppm)	Nuclear Enlargement	Cytoplasmic Eosinphilia	Hypertrophy	Peroxisomal Staining
Male/	0	!	!	!	+
	2000	++	++	++	++
Female	0	!	!	!	+
	2000	++	++	++	++
Male	500 NAF	++	++	++	+++
Female	500 NAF	++	++	++	++

[1] Data summarized from Table 12 of MRID No. 452383904.

3.) Measurement of Biochemical Markers for Peroxisome Proliferation in Primary Rat Hepatocytes (MRID No.: 45283902): In this study, primary cultures of rat hepatocytes were exposed to PPG-844 (lactofen, 99.8%) or five of its principal animal metabolites (structures Figure 2): PPG-947 (desethyl lactofen 98.9% purity), PPG-847 (acrifluorfen, 100% purity), PPG-1576 (amino lactofen, 95.6% purity), and PPG-2053 (amino acifluorfen, 94.6% purity), PPG-2838 (desethyl amino lactofen, 70.6% purity). Because of the low purity of PPG 2838, it was excluded from the definitive study. CN-insensitive palmitoyl CoA oxidase activity, a biomarker for peroxisome proliferation was measured after 68 hr of exposure. Solvent (DMSO) and positive (clofibrate) were also evaluated.

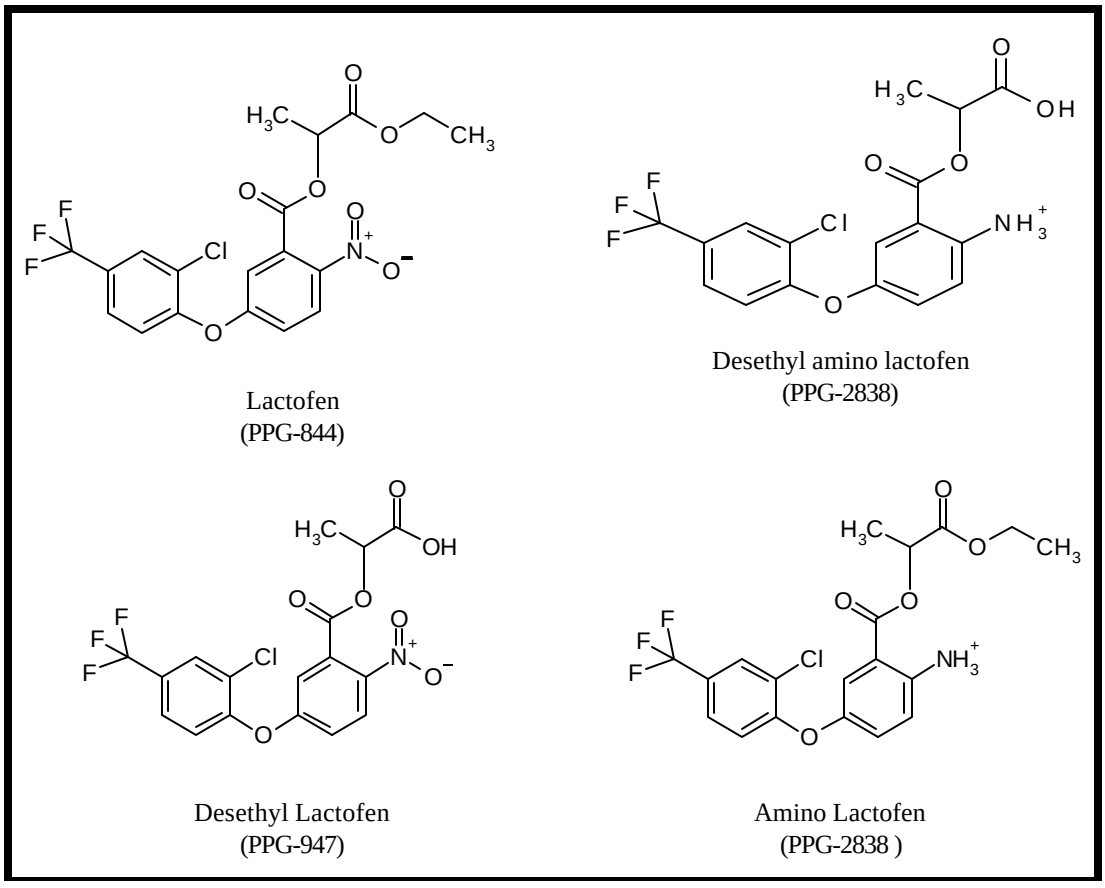
Additionally, control, lactofen, metabolite and clofibrate-treated hepatocyte were examined using an electron microscope.

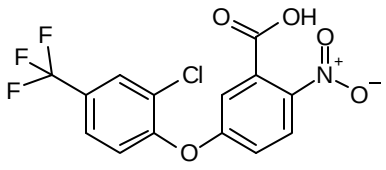
CN-insensitive palmitoyl CoA oxidase data are summarized in Table 8. Of the test

chemicals evaluated, treatment with lactofen resulted in greater than 5-fold, concentration-dependent increase in palmitoyl CoA oxidase activity compared to the solvent control. the highest increase for the metabolites was approximately 3-fold. Decending order of potency, based on palmitoyl CoA oxidase activity, was PPG-844, -947, -847, -1576 and -2053. Clofibrate produced significant increases (6.9 to 7.7-fold) in enzyme activity at both of the concentrations test.

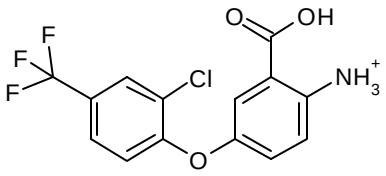
Electron micrographs of lactofen and clofibrate-treated hepatocytes revealed an increased numbers of dense, anucleoide peroxisomes compared to the solvent control. Additionally, lactofen-treated hepatocytes showed increased amounts of cytoplasmic glycogen aggregates. Treatment with the metabolites did not cause any increase in the number of peroxisomes relative to the control.

Figure 2: Structures of Lactofen and Primary Metabolites





Acifluorfen
(PPG-847)



Amino Acifluorfen
(PPG-2053)

Table 8: Effect of Lactofen and Metabolites on CN-Insensitive Palmitoyl CoA Oxidase Activity (nmol/min/mg protein) in Primary Rat Hepatocytes

Addition	Concentration, mM			
	0.003	**0.01**	**0.03**	**0.10**
PPG-844 (Lactofen)	1.55*	2.47*	NT	2.63*
PPG-947	1.61*	1.68*	NT	1.74*
PPG-847	NT [2]	1.35*	1.35*	1.75*
PPG-1576	NT	1.26*	0.74[3]	1.57[3]
PPG-2053	NT	0.86	0.98[3]	1.46*
Controls				
Solvent Control (DMSO)		0.50		
Positive Control Clofibrate, 0.16 mM		3.46*		
Clofibrate, 0.50 mM		3.86*		

[1] Data summarized from Table 2 of MRID No. 45283902.
[2] NT = not tested
[3] Mean of two observations, significance not determined.
* Significantly different from solvent control, p # 0.05.

IV. Other Modes of Action

Studies with transgenic mouse confirmed that essentially all of the of the effects of PPs in rodent liver are mediated by PPARá.

V. Recommendations to the MTARC

Based on the weight-of-the-evidence of the toxicity database, there are sufficient data to classify lactofen as a non-genotoxic hepatocarcinogen in rodents with peroxisome proliferation being a plausible mode of action.

VI. Relevancy to Humans

The human relevancy of the role of peroxisome proliferators was not addressed, and will be deferred pending a proposed ILSI evaluation of pertinent data.

VI. References

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